Neoamphimedine: A New Pyridoacridine Topoisomerase II Inhibitor Which Catenates DNA

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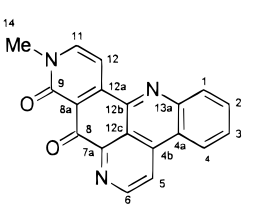
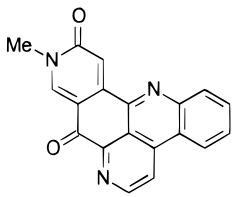
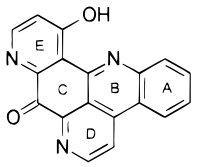
The pyridoacridines constitute an important class of marine metabolites which have exhibited significant cytotoxicity. These metabolites have been isolated predominantly from tunicates and sponges but have also been reported from an anemone and a prosobranch.[4] We report here the isolation of the pyridoacridines neoamphimedine (**1**) and amphimedine (**2**), from *Xestospongia* sp. from the Philippines and *Xestospongia* cf. *carbonaria*. from Micronesia. Amphimedine has been reported previously.[5]

The sponge *Xestospongia* sp., collected from Surigao, Philippines, was extracted with MeOH and the extract partitioned with hexane and then $CHCl_3$. The $CHCl_3$ partition extract was chromatographed on a column of Sephadex LH-20 using MeOH as eluting solvent. The first yellow band that eluted contained neoamphimedine (**1**). The succeeding yellow-to-peach band was further chromatographed by silica vacuum liquid chromatography using 15% MeOH−$CHCl_3$ as eluent, yielding two yellow bands, the first band being amphimedine (**2**) and the second neoamphimedine (**1**).

The sponge *X.* cf. *carbonaria*, collected from Palau, was extracted with MeOH/CH_2Cl_2. The resulting crude extract was partitioned between H_2O and 9:1 CH_2Cl_2/MeOH. The organic layer was further purified by chromatography on silica gel and chromatography on Sephadex LH-20, yielding neoamphimedine (**1**) and amphimedine (**2**).

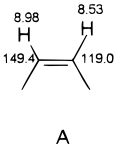






High-resolution EIMS of amphimedine gave a base peak at *m*/*z* 313.08831, corresponding to a molecular formula of $C_{19}H_{11}N_3O_2$ (Δ 3.81 mmu). The chemical formula, chemical shifts, and proton coupling pattern for this compound appeared the same as for amphimedine isolated by Schmitz and co-workers.[5] Comparison of the spectral data with those provided by Schmitz showed that the two compounds were identical.

The high-resolution fast atom bombardment (FAB) mass spectrum of neoamphimedine (**1**) required a molecular formula of $C_{19}H_{11}N_3O_2$, isomeric with amphimedine (**2**). ^{1}H NMR spectra of both compounds showed signals for eight aromatic protons and one methyl singlet, the major difference being the presence of two *ortho* coupled (J = 8 Hz) protons in neoamphimedine (**1**) as compared to two isolated singlets in amphimedine (**2**). Decoupling and HMQC experiments carried out on neoamphimedine indicated the three spin systems



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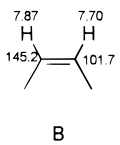

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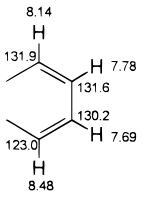

C

The chemical shifts for the protons in spin systems A and C in neoamphimedine (**1**) are nearly identical with those of meridine (**3**),[6] suggesting the same gross structure, differing only in ring E.

GHMBC data further extended these partial structures as shown by the long-range heteronuclear correlations Insertion of a nitrogen between carbons at δ 146.2 and 145.2 and between the carbons at δ 146.5 and 149.4 accounts for their downfield shifts. Ring E of neoamphimedine (**1**) was established by the three-bond correlations shown by H-11, H-12, and the methyl protons. A carbonyl at δ 179.8 is the only one still unassigned, and the only remaining position is between the carbons at δ 118.9 and 146.5. Thus, neoamphimedine (**1**) has the same skeleton as meridine for rings A−D but differs only in ring E.

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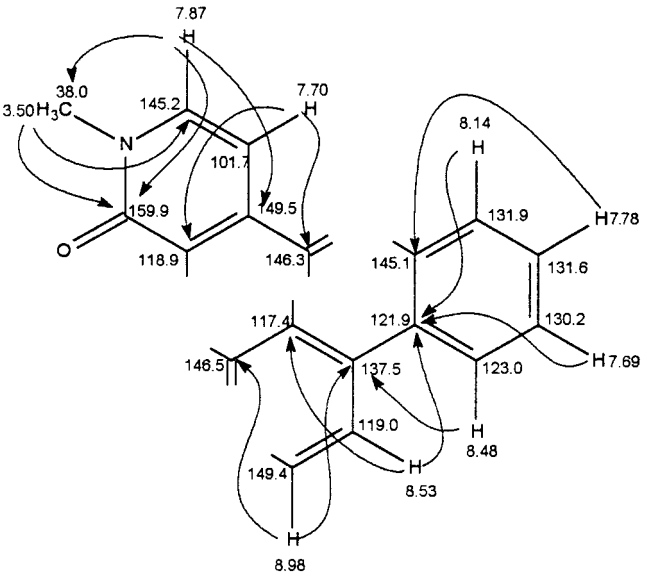

Neoamphimedine (**1**) was cytotoxic to normal CHO AA8 cells with an IC_{50} of 2 μg/mL. Quantitative DNA cleavage assays performed on neoamphimedine (**1**) revealed it stimulates topoisomerase II dependent cleavage 3% above controls compared to etoposide, which stimulates 38% cleavage at the same concentration. No stimulation of DNA cleavage was seen with amphimedine in the presence of topoisomerase II. A noteworthy finding from these cleavage assays is that neoamphimedine (**1**) has the novel ability to stimulate topoisomerase II to catenate DNA to a high-molecular-weight complex (8% of the DNA was catenated).[7] This complex was heat- and detergent-stable. The DNA complex was not due to protein or chemical cross-linking with plasmid DNA. DNA catenation as the form of this complex was confirmed by reacting it with the restriction enzyme Sal I. Sal I has a unique restriction site in the substrate MP-19 DNA. When reacted with Sal I, only one prominent species of DNA is visible on the gel, corresponding to linear DNA. Transmission electron microscopy analysis of the DNA was consistent with this hypothesis.

Few molecules are known to induce topoisomerase II to catenate DNA. Poly(ethylene glycol) and histones and histone-like proteins all carry this rare distinction.[8−11] Topoisomerase II−DNA catenation can be facilitated by neutralization of charge or ionic conditions allowing compaction of DNA.[11] In this study, *in vitro* catenation of supercoiled, plasmid DNA induced by topoisomerase II and neoamphimedine (**1**) is reported, as confirmed by electrophoretic and TEM techniques. Catenation is achieved by strand breakage, strand passage, and ligation of the DNA by topoisomerase II. The fact that catenation of mp 19 DNA by neoamphimedine requires topoisomerase II is consistent with this model. It is hypothesized that neoamphimedine is a DNA-active molecule that alters DNA structure in such a way that it facilitates catenation by topoisomerase II.

Table 1. NMR Data for Neoamphimedine (1)

	2:1 $CDCl_3/CD_3OD$[a]			2:1 TFA-*d*/$CDCl_3$[b]	
C no.	δ(C)	δ(H)	GHMBC, C no.	δ(C), mult	δ(H) (multiplicity, intensity, *J* in Hz)
1	131.9	8.14	3,4a	134.3, d	8.70 (d, 1H, 8.0)
2	131.6	7.78	4, 13a	137.5, d	8.38 (t, 1H, 8.0)
3	130.2	7.69	1, 4a	134.3, d	8.24 (t, 1H, 8.0)
4	123.0	8.48	2, 4b, 13a	125.7, d	8.95 (d, 1H, 8.0)
4a	121.9			120.7, d	
4b	137.5			145.6, s	
5	119.0	8.53	4a, 6, 12c	125.5, d	9.48 (d, 1H, 6.6)
6	149.4	8.98	4b, 5, 7a	140.3, d	9.33 (d, 1H, 6.6)
7a	146.5			138.7, s	
8	179.8			175.5, s	
8a	118.9			116.7, s	
9	159.9			161.9, s	
11	145.2	7.87	8a, 9, 12a, 14	144.2, d	8.48 (d, 1H, 8.0)
12	101.7	7.70	8a, 11	108.1, d	8.51 (d, 1H, 8.0)
12a	149.5			151.5, s	
12b	146.2			145.0, s	
12c	117.5			117.9, s	
13a	145.2			148.0, s	
14	38.0	3.50	8, 11	40.2, s	4.06 (s, 3H)

[a] 500 MHz NMR. [b] 360 MHz NMR.

Drugs which induce catenation of tumor cell DNA could serve as effective tumoricidal agents, possibly by interfering with replication and segregation of chromosomes. Neoamphimedine (**1**) may have utility as an antineoplastic agent because of its novel mechanism of cytotoxicity.

Experimental Section

Extraction and Isolation of Neoamphimedine (1) and Amphimedine (2). The sponge was collected by SCUBA at a depth of 8 ft at Bucas Grande, Surigao, Philippines, on March 10, 1997. The sponge was identified as *Xestospongia* sp. by Mary Kay Harper of the Scripps Institution of Oceanography, La Jolla, CA. A voucher sample of this sponge is kept at the Marine Science Institute, University of the Philippines, Diliman, Quezon City, Philippines, and at the University of Utah, Salt Lake City, UT. The sponge (95.2 g) was extracted with MeOH. The crude MeOH extract was partitioned with hexane and then $CHCl_3$. The $CHCl_3$ layer (111.2 mg) was then chromatographed on a column of Sephadex LH-20 using MeOH as the eluting solvent, giving pure **1** as the first yellow band that eluted out and a yellow-peach band, which consisted of a mixture of **1** and **2**. This mixture was subjected to vacuum liquid chromatography on silica gel using 15% MeOH−85% $CHCl_3$ as eluent, resulting in two yellow bands. The first yellow band consisted of **2,** and the second yellow band to elute was **1**.

Xestospongia cf. *carbonaria* (voucher number 81−128) was collected by hand during March 1981 from a small marine lake (−3 m), located on Urukthapel Island, Republic of Palau. The sample was stored in MeOH at −10 °C until workup. The sponge was identified by Dr. Rob van Soest, Instituut voor Taxonomische Zoologie, Amsterdam, The Netherlands.[12] The MeOH was decanted from the sponge, which was extracted three more times with 1:1 MeOH/CH_2Cl_2 (1 L). The combined organic extracts were evaporated *in vacuo* to give a crude yellow extract. The crude extract was partitioned between H_2O and 9:1 CH_2Cl_2/MeOH to give 3 g of organic extract. The organic extract was chromatographed on silica gel using an increasing gradient of MeOH in CH_2Cl_2 and then rechromatographed on Sephadex LH-20 using 1:1 MeOH/CH_2Cl_2 as eluent to give, in order of elution,

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(12) The sponge was described as having an ectosomal skeleton with a tangential unispicular (frequently irregular) arrangement; the choanosomal skeleton was made up of isotropically arranged bundles, and the spicules were 150−500 mm in length. Dr. Rob van Soest compared the sample with the voucher sample of *Amphimedon* sp. (USNM 31765) from which amphimedine was previously reported,[5] found the two samples to be identical, and classified both as *Xestospongia* cf. *carbonaria*. A voucher specimen of 81-128 (ZMA POR.8420) has been deposited in the Zoological Museum at the University of Amsterdam.

amphimedine (**2**; 60 mg, 0.19 wt % of crude extract) and neoamphimedine (**1**; 90 mg, 0.28 wt % of crude extract).

Neoamphimedine (1): yellow solid, mp >300 °C; HR FABMS obsd *m*/*z* 314.0948, calcd for $C_{19}H_{12}N_3O_2$ ($(M + H)^+$) *m*/*z* 314.0930; FTIR (KBr) 3100−3000, 1688, 1621, 1606, 1593, 1528, 1340, 1071, 1001, 928, 840, 799, 775, 758, 728 cm^{-1}; UV (EtOH) 205 nm (ϵ 26 370), 226 nm (ϵ 23 740), 278 nm (ϵ 16 870), 371 nm (ϵ 10 190); ^1H NMR ($CDCl_3$)) 8.36 (d, H-1), 7.96 (t, H-2), 7.86 (t, H-3), 8.62 (d, H-4), 8.56 (d, H-5), 9.30 (d, H-6), 7.83 (d, H-11), 7.81 (d, H-12), 3.70 (s, H-14), for other resonances, see Table 1; ^{13}C NMR see Table 1.

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Supporting Information Available: ^1H, ^{13}C, HMQC, and HMBC NMR spectral data for neoamphimedine (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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